Safety and immunogenicity of the novel tuberculosis vaccine ID93 + GLA-SE in BCG-vaccinated healthy adults in South Africa: a randomised, double-blind, placebo-controlled phase 1 trial

Adam Penn-Nicholson, Michele Tameris*, Erica Smit, Tracey A Day, Munyaradzi Musvosvi, Lakshmi Jayashankar, Julie Vergara, Simbarashe Mabwe, Nicole Bilek, Hendrik Geldenhuys, Angelique Kany-Kany Luabeya, Ruth Ellis, Ann M Ginsberg, Willem A Hanekom, Steven G Reed, Rhea N Coler, Thomas J Scriba*, Mark Hatherill*, and the TBVPX-114 study team†*

Summary

Background A vaccine that prevents pulmonary tuberculosis in adults is needed to halt transmission in endemic regions. This trial aimed to assess the safety and immunogenicity of three administrations at varying doses of antigen and adjuvant of an investigational vaccine (ID93 + GLA-SE) compared with placebo in previously BCG-vaccinated healthy adults in a tuberculosis endemic country.

Methods In this randomised, double-blind, placebo-controlled phase 1 trial, we enrolled HIV-negative, previously BCG-vaccinated adults (aged 18–50 years), with no evidence of previous or current tuberculosis disease, from among community volunteers in the Worcester region of Western Cape, South Africa. Participants were randomly assigned to receive varying doses of ID93 + GLA-SE or saline placebo at day 0, day 28, and day 112. Enrolment into each cohort was sequential. Cohort 1 participants were *Mycobacterium tuberculosis* uninfected (as defined by negative QuantiFERON [QFT] status), and received 10 μg ID93 plus 2 μg GLA-SE, or placebo; in cohorts 2–4, QFT-negative or positive participants received escalating doses of vaccine or placebo. Cohort 2 received 2 μg ID93 plus 2 μg GLA-SE; cohort 3 received 10 μg ID93 plus 2 μg GLA-SE; and cohort 4 received 10 μg ID93 plus 5 μg GLA-SE. Dose cohort allocation was sequential; randomisation within a cohort was according to a randomly-generated sequence (3 to 1 in cohort 1, 5 to 1 in cohorts 2–4). The primary endpoint was safety of ID93 + GLA-SE as defined by solicited and unsolicited adverse events up to 28 days after each study injection and serious adverse events for the duration of the study. Specific immune responses were measured by intracellular cytokine staining, flow cytometry, and ELISA. All analyses were done according to intention to treat, with additional per-protocol analyses for immunogenicity outcomes. This trial is registered with ClinicalTrials.gov, number NCT01927159.

Findings Between Aug 30, 2013, and Sept 4, 2014, 227 individuals consented to participate; 213 were screened (three participants were not included as study number was already met and 11 withdrew consent before screening occurred, mostly due to relocation or demands of employment). 66 healthy, HIV-negative adults were randomly allocated to receive the vaccine (n=54) or placebo (n=12). All study participants received day 0 and day 28 study injections; five participants did not receive an injection on day 112. ID93 + GLA-SE was well tolerated; no severe or serious vaccine-related adverse events were recorded. Vaccine dose did not affect frequency or severity of adverse events, but mild injection site adverse events and flu-like symptoms were common in *M tuberculosis*-infected participants compared with uninfected participants. Vaccination induced durable antigen-specific IgG and Th1 cellular responses, which peaked after two administrations. Vaccine dose did not affect magnitude, kinetics, or profile of antibody and cellular responses. Earlier boosting and greater T-cell differentiation and effector-like profiles were seen in *M tuberculosis*-infected than in uninfected vaccinees.

Interpretation Escalating doses of ID93 + GLA-SE induced similar antigen-specific CD4-positive T cell and humoral responses, with an acceptable safety profile in BCG-immunised, *M tuberculosis*-infected individuals. The T-cell differentiation profiles in *M tuberculosis*-infected vaccinees suggest priming through natural infection. While cohort sample sizes in this phase 1 trial were small and results should be interpreted in context, these data support efficacy testing of two administrations of the lowest (2 μg) ID93 vaccine dose in tuberculosis endemic populations.

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*Authors contributed equally

† Study team members listed in the appendix

South African Tuberculosis Vaccine Initiative (SATVI), Institute of Infectious Disease and Molecular Medicine, and Division of Immunology, Department of Pathology, University of Cape Town, Cape Town, South Africa (A Penn-Nicholson PhD, M Tameris MBChB, E Smit BTech, M Musvosvi PhD, S Mabwe MSc, N Bilek PhD, H Geldenhuys MBChB, A Kany-Kany Luabeya MBChB, Prof W A Hanekom FCP[SA], T J Scriba PhD, Prof M Hatherill MD)**; Infectious Disease Research Institute, Seattle, WA, USA** (T A Day PhD, L Jayashankar PhD, J Vergara BA, Prof S G Reed PhD, Prof R N Coler PhD)**; and Aeras, Rockville, MD, USA** (R Ellis MD, A M Ginsberg MD)

Correspondence to:
Prof Mark Hatherill, Faculty of Health Sciences, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, 7925 Cape Town, South Africa
mark.hatherill@uct.ac.za

See Online for appendix

Introduction

The only currently licensed tuberculosis vaccine BCG induces effector memory T-cell responses that mainly express IFNγ. These Th1 responses are believed to be important for protective immunity,[1] yet BCG exhibits highly variable efficacy against pulmonary tuberculosis (ranging from 0 to 80%).[2] Efficacy is maximal in young children who have not been exposed to any type of

Research in context

Evidence before this study

Tuberculosis is the world's number one cause of infectious disease mortality and 23% of the global population is estimated to be infected with the causative agent, *Mycobacterium tuberculosis*. An effective tuberculosis vaccine that halts progression from infection to disease, preventing onward transmission of *M tuberculosis*, is needed urgently. We searched PubMed and Google Scholar for studies of tuberculosis vaccines published in English up to Jan 27, 2018, with the terms "tuberculosis vaccine", "adjuvant", "subunit vaccine", "vaccine AND QuantiFERON", and "vaccine AND latent TB infection". Many publications were studies of tuberculosis vaccine candidates in clinical development and other subunit and adjuvant vaccines targeting other diseases. Findings from the most relevant articles were considered with our findings. In addition, we reviewed unpublished data from a previous phase 1 study in which ID93 + GLA-SE had an acceptable safety and immunogenicity profile in BCG-naive, *M tuberculosis*-uninfected, healthy adults (NCT01599897). Other ongoing studies include a phase 1 trial comparing ID93 with either GLA-SE or a liposomal adjuvant formulation (NCT02508376) and a phase 2a trial administering ID93 + GLA-SE to tuberculosis patients after treatment completion (NCT02465216).

Added value of this study

This is the first report of safety and immunogenicity of ID93 + GLA-SE in human beings. Our findings showed an acceptable safety profile in both *M tuberculosis*-infected and uninfected BCG-vaccinated adults in South Africa. Earlier boosting and greater T-cell differentiation and effector-like immune profiles were recorded in *M tuberculosis*-infected compared with uninfected vaccinees, suggesting priming through natural *M tuberculosis* infection. Vaccination elicited high-magnitude and sustained CD4-positive T-cell and antibody responses, with little effect of dose on magnitude or kinetics of antibody and cellular responses. Dose evaluation in *M tuberculosis*-infected populations is crucial for planned efficacy trials in endemic countries.

Implications of all the available evidence

This study supports progression of ID93 + GLA-SE to efficacy trials of two administrations of the lowest (2 μg) ID93 vaccine dose in tuberculosis endemic populations.

mycobacteria, and poorest in *M tuberculosis*-infected adults,[3] hence the practice of universal infant BCG vaccination in countries endemic for tuberculosis.[4]

Pulmonary tuberculosis in adults is primarily responsible for aerosol *M tuberculosis* transmission and, in countries with high burdens of tuberculosis, most adults are infected with *M tuberculosis* and therefore at risk of disease.[5] Thus, a new effective vaccine that protects both *M tuberculosis*-infected and uninfected adults from tuberculosis disease is needed.[5] *M tuberculosis* infection is characterised by a spectrum that spans quiescence, subclinical disease through to active tuberculosis disease.[6,7] Different *M tuberculosis* proteins are expressed and recognised by the immune system during the different stages of this infection spectrum.[8,9] We proposed that a vaccine should ideally induce immune responses against a range of these antigens, irrespective of the infection stage. The phenotype, functional capacity, and tissue location of antigen-specific T cells are also likely to be crucial in preventing disease.[10] Additionally, recent findings suggest that antibodies might contribute to infection outcome, including protection against disease.[11]

ID93 + GLA-SE is a novel subunit tuberculosis vaccine candidate. The recombinant fusion protein ID93 comprises four antigens associated with virulence (Rv2608, Rv3619, and Rv3620) or latency (Rv1813).[12] These antigens elicited dominant Th1 responses associated with reduced bacterial burden in animal models.[12] ESX-1 family members Rv3619 and Rv3620 are unique to *M tuberculosis* with no homologues in *Mycobacterium bovis* or BCG, whereas Rv1813 and Rv2608 are expressed by both *M bovis* and BCG.[13,14] ID93 is formulated in a synthetic toll-like receptor 4 agonist in a stable oil-in-water emulsion known as glucopyranosyl lipid A stable emulsion (GLA-SE) formulation.[15,16] Using mice, guinea pigs, and non-human primates, we have shown that ID93 + GLA-SE is efficacious, providing prophylactic protection against challenge with laboratory adapted, drug-resistant or hypervirulent Beijing clinical *M tuberculosis* isolates.[17–19] ID93 + GLA-SE also showed utility as a therapeutic vaccine in mice and non-human primates, where vaccination during or at the end of chemotherapy improved outcome over antibiotics alone,[20] and allowed the duration of antibiotic treatment to be reduced by 30% in mice. Therapeutic efficacy of ID93 + GLA-SE is associated with enhanced Th1 responses, improved *M tuberculosis* clearance, and reduced pulmonary inflammation.[20] As non-human primates recapitulate many aspects of human tuberculosis susceptibility and disease, the protection afforded by ID93 + GLA-SE in this model shows promise for efficacy in human beings. Further, the vaccine had an acceptable safety and immunogenicity profile in BCG-naive, *M tuberculosis*-uninfected, healthy adults (NCT01599897). Using safety results from this phase 1 trial, this study was designed to select a dose of both antigen and adjuvant intended for use in a tuberculosis endemic setting, such as South Africa. Ongoing studies include a phase 1 trial comparing ID93 with either GLA-SE or a liposomal adjuvant formulation (NCT02508376) and a phase 2a trial administering ID93 + GLA-SE to patients with tuberculosis after treatment completion

(NCT02465216). In this first report of clinical safety, reactogenicity, and immunogenicity of ID93+GLA-SE, we compare escalating doses of both vaccine antigen and adjuvant, versus placebo control, administered to BCG-vaccinated *M tuberculosis*-uninfected or infected adults in a tuberculosis endemic setting.

Methods

Study design and participants

In this single-centre randomised, double-blind, placebo-controlled phase 1 trial, we enrolled HIV-negative, previously BCG-vaccinated adults (aged 18–50 years), with no evidence of previous or current tuberculosis disease, from among community volunteers in the Worcester region of Western Cape, South Africa. The appendix details inclusion and exclusion criteria and study procedures. QuantiFERON TB Gold In-Tube test (QFT) was used to define *M tuberculosis* infection according to the manufacturer's instructions (QIAgen). Written informed consent was obtained from all participants. The protocol was approved by the Medicines Control Council of South Africa and the Human Research Ethics Committee of the University of Cape Town.

Randomisation and masking

Enrolment into one of four cohorts occurred sequentially. First, only QFT-negative participants were enrolled (cohort 1). Thereafter, community volunteers (whether QFT-positive or QFT-negative) were enrolled into successive cohorts of escalating doses of both ID93 and GLA-SE (cohorts 2–4), pending favourable review of safety data though day 35 from preceding cohorts. Dose selection was informed by previous data from BCG naive, *M tuberculosis*-uninfected adults (NCT01599897). Cohort 1 received 10 μg ID93 plus 2 μg GLA-SE. Cohort 2 received 2 μg ID93 plus 2 μg GLA-SE; cohort 3 received 10 μg ID93 plus 2 μg GLA-SE; and cohort 4 received 10 μg ID93 plus 5 μg GLA-SE. Participants were randomly assigned within each dose cohort to placebo or ID93+GLA-SE at a ratio of 1 to 3 (cohort 1) or 1 to 5 (cohorts 2–4) via a validated Interactive Voice Response System/Interactive Web Response System (IVRS/IWRS), whereby the investigator randomised the participant through the IVRS/IWRS and the study vaccine manager consulted the randomisation–unmasked response notification. The unmasked study pharmacist prepared the vaccine in a masked syringe (semi-transparent tape) and it was administered by an unmasked vaccinating nurse who was not involved in participant follow up and safety assessment. Both participants and clinical and laboratory staff involved in assessing outcomes or data analysis were masked to study group assignment. No incidents of unintended unmasking to group assignment were reported.

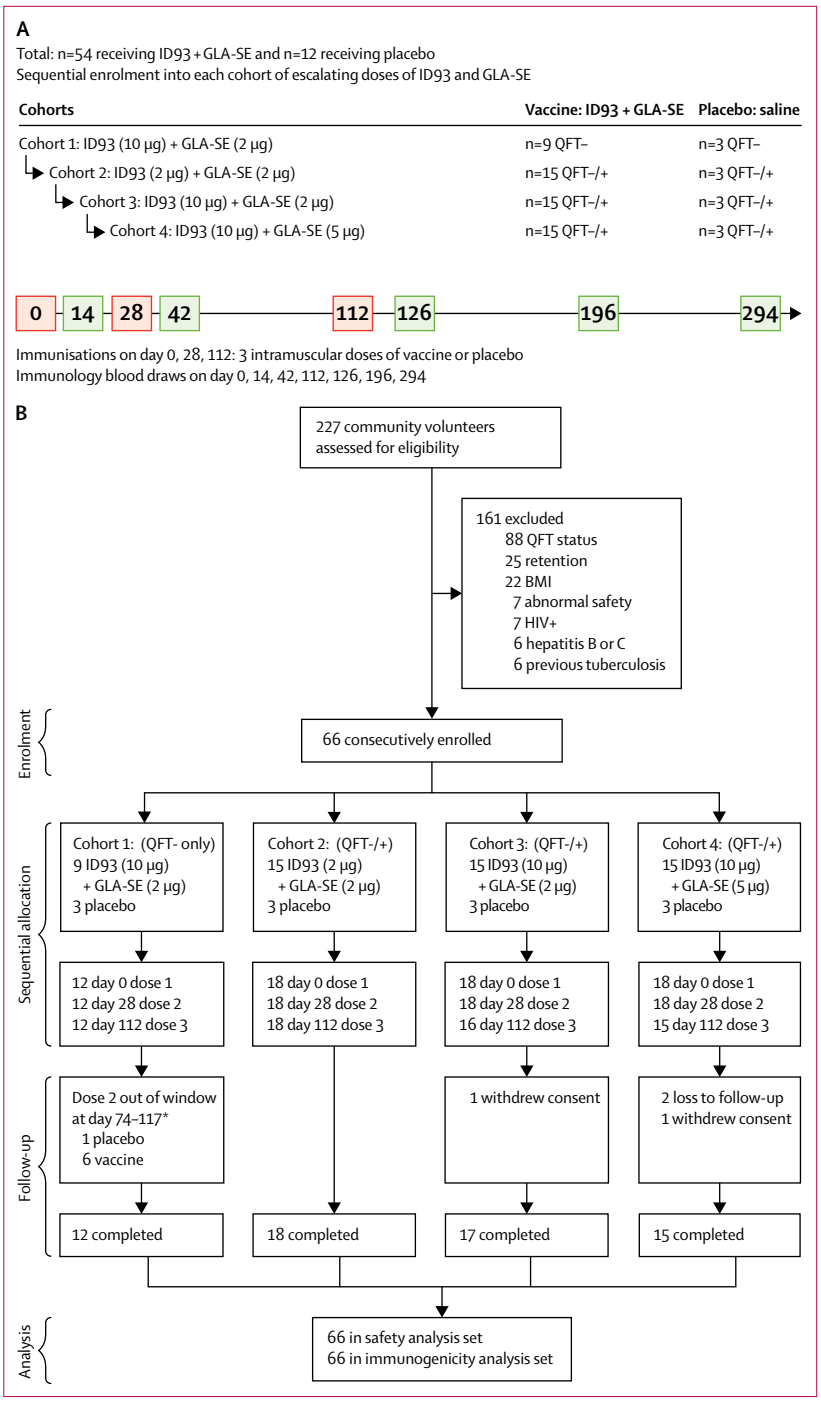

Figure 1: **(A) Study design schematic and (B) trial profile**
Participants missing the third injection either withdrew consent, were lost to follow up, were pregnant, or missed the injection visit. Participants were in follow up for 6 months after the third injection. The safety analysis set is defined as all participants who received at least one study injection; the immunogenicity analysis set is defined as all participants who received at least one study injection, and who did not have a treatment deviation of receiving a different dose than that to which they were randomly assigned. *Any samples collected within the protocol specified intervals after an out-of-window vaccination visit were analysed as intention to treat.

Procedures

ID93+GLA-SE was developed and manufactured by the Infectious Disease Research Institute (Seattle, WA, USA). On each of study days 0, 28, and 112, participants received a 0·5 mL intramuscular injection of either saline placebo or ID93+GLA-SE at doses escalating with each sequentially enrolled cohort (figure 1 and appendix),

pending favourable review of safety data through day 35 from the preceding cohort.

All participants were followed up for 6 months after the final study injection. Injection site reactions and other adverse events were assessed 1, 3, 7, 14, and 28 days after each study injection. Laboratory assessments were done 7 days after each injection; serious adverse events were recorded throughout 6-month follow-up.

Phlebotomy for immunogenicity was done on study days 0, 14, 42, 112, 126, 196, and 294. Serum IgG ELISA was done with recombinant ID93 and each fusion-protein antigen component to measure antibodies. Whole blood intracellular cytokine staining (ICS) was performed to measure T-cell responses as previously described,[21] with 15mer peptides spanning Rv1813, Rv2608, Rv3619, or Rv3620 (1 μg/ml per peptide) phytohaemagglutinin (PHA; 5 μg/mL Bioweb), or media alone. Details of the assay procedures are provided in the appendix.

Outcomes

The primary outcome was safety and reactogenicity, assessed by solicited local adverse events (injection site erythema, pain, and swelling), solicited systemic adverse events (chills, fatigue, headache, myalgia, pyrexia, arthralgia, decreased appetite, hives), and unsolicited adverse events within 28 days of vaccination, and serious adverse events throughout the entire study. Events were graded as mild, moderate, or severe; association with vaccination was assessed and reported. Unsolicited local and systemic adverse events were evaluated and reported for all participants. We scored adverse event intensities on a scale based on the US Food and Drug Administration (FDA) guidelines for healthy adult and adolescent participants enrolled in preventative vaccine clinical trials (September, 2007). The secondary outcome was assessment of cellular and humoral immunogenicity induced by ID93+GLA-SE, as defined by ELISA and whole blood ICS assay.

For **FDA guidelines** see https://www.fda.gov/downloads/BiologicsBloodVaccines/ucm091977

Together, these data were used to select a dose of ID93+GLA-SE for future clinical trials in a tuberculosis-endemic setting in which vaccine recipients are likely to have previously been vaccinated with BCG and might also harbour *M tuberculosis* infection.

Statistical analysis

The sample size for this study was selected as adequate to detect frequent adverse events. In view of a total sample size of 54 participants receiving ID93+GLA-SE at any dose level, there was an 80% chance of recording at least one adverse event that occurred at a proportion of 3%. In each cohort of 15 vaccine recipients, there was an approximately 80% probability of detecting at least one adverse event that occurred at a proportion of 10%. Alternatively, if no events were to be recorded in vaccine recipients, the upper bound of the one-sided 95% confidence interval (CI) on the proportion of event occurrence was estimated at approximately 5·4%. We performed descriptive summaries of safety and demographic data and calculated exact binomial CIs for high-level safety data. We did not do any formal statistical comparisons because of small group sizes. This study was designed as a preliminary assessment of the vaccine safety profile at various dose levels. Statistical analysis was done with SAS (version 9.1.3 or later).

ICS assay data analysis was done with FlowJo software (version 9.9, TreeStar) using a pre-designed template. The appendix provides details of exclusion criteria for flow cytometry data. Flow cytometric analysis of specific cytokine-expressing T cells was reported after subtraction of frequencies of cytokine-expressing T cells in the negative (unstimulated) control. Antibody endpoint titres were calculated using optical density (OD) 450 nm minus OD 570 nm values for interpolating unknowns from the last OD value greater than the threshold given by naive sera using a 4-parameter logistic model (Parameter 208) XL-Fit software as a Microsoft Excel add-in.

Statistical analysis and graphical representation of data were done using GraphPad Prism (version 7.0). We calculated relative risk with the Katz log method. For analysis of longitudinal immune responses, we calculated the area under the curve (AUC) for each individual using the trapezoid rule as previously described.[22] Where comparative analyses involved more than two groups, we either used the Kruskal-Wallis (between groups) or Friedman (within a group) tests. If a p value was lower than 0·05 from these tests, we did a post-test analysis using Mann-Whitney *U* (between groups) or Wilcoxon matched pair tests (within a group). When comparing more than two groups, we accounted for multiple testing by adjusting p-values using the Bonferroni method (eg, where $p<0·0125$ was considered significant for four tests). IFNγ, IL-2 and TNF-α co-expressing CD4-positive T cell responses were quantified by

	Placebo group (saline; n=12)	Cohort 1 (10 μg ID93 + 2 μg GLA-SE; n=9)	Cohort 2 (2 μg ID93 + 2 μg GLA-SE; n=15)	Cohort 3 (10 μg ID93 + 2 μg GLA-SE; n=15)	Cohort 4 (10 μg ID93 + 5 μg GLA-SE; n=15)
Baseline QFT status					
Negative	4 (33%)	9 (100%)	1 (7%)	1 (7%)	1 (7%)
Positive	8 (67%)	0	14 (93%)	14 (93%)	14 (93%)
Age (years)	28 (20,47)	20 (19, 22)	25 (18, 47)	27 (20, 42)	24 (18, 46)
Sex					
Female	11 (92%)	9 (100%)	11 (73%)	14 (93%)	10 (67%)
Male	1 (8%)	0	4 (27%)	1 (7%)	5 (33%)
Ethnic origin					
Black	3 (25%)	6 (67%)	5 (33%)	6 (40%)	4 (27%)
White	1 (8%)	0	0	0	0
Mixed race	8 (67%)	3 (33%)	10 (67%)	9 (60%)	11 (73%)
Baseline BMI (kg/m^2)	25·5 (23, 31)	22·9 (19, 30)	26·8 (19, 32)	28·1 (20, 33)	23·8 (20, 33)

Data are n (%) or median (min, max). QFT=QuantiFERON.

Table 1: **Baseline demographics**

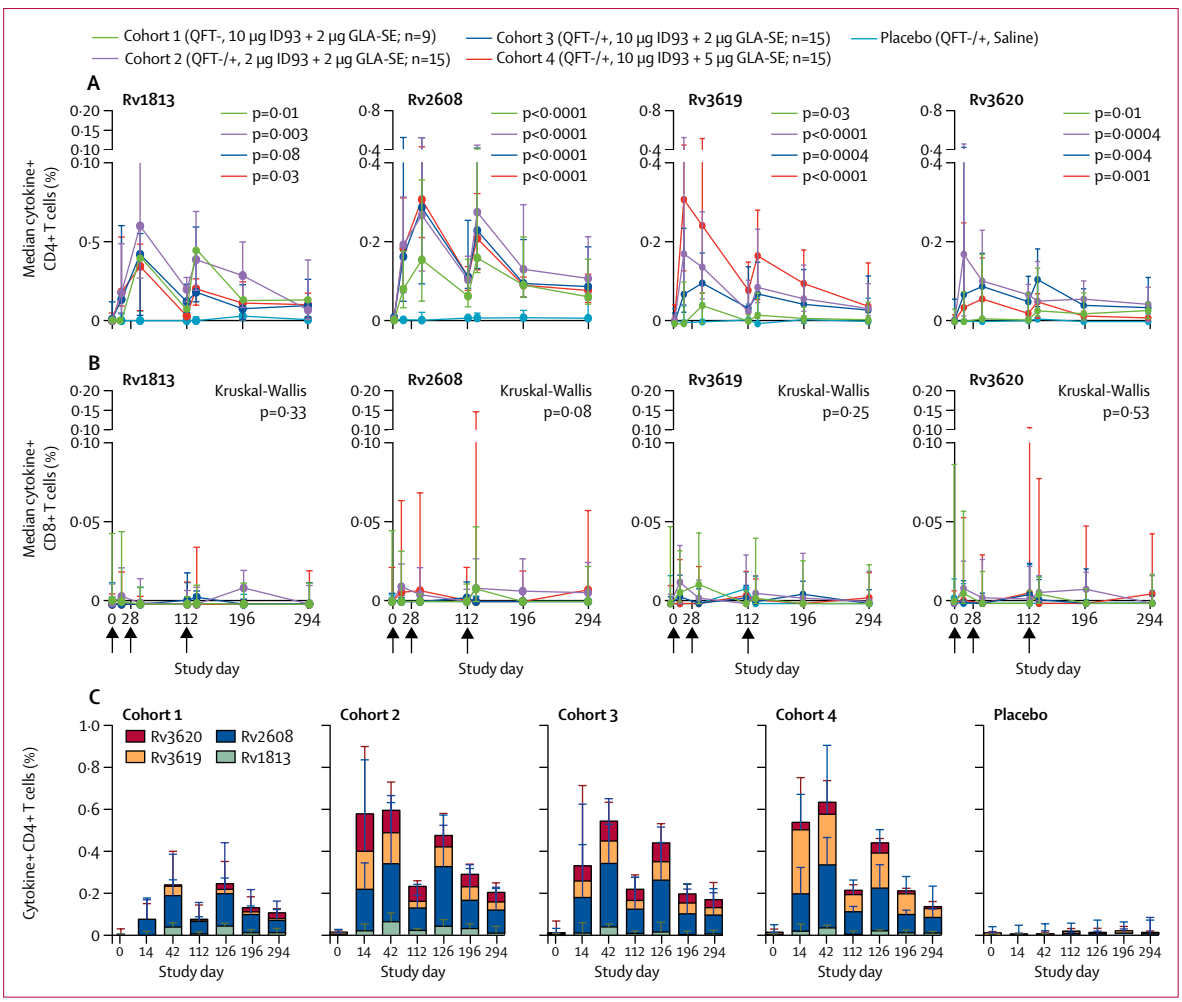

Figure 2: **Median total T-cell responses induced by vaccination, stratified according to study cohort**
(A) Median total peptide-pool Rv1813, Rv2608, Rv3619, and Rv3620-specific CD4-positive T-cell responses in vaccinees before and after vaccination, measured by whole blood intracellular cytokine staining. (B) Median total peptide-pool Rv1813, Rv2608, Rv3619, and Rv3620-specific CD8-positive T-cell responses. For all graphs, error bars represent IQR, and arrows indicate days of vaccine or placebo administration. Background values (unstimulated) were subtracted. Mann-Whitney p values are shown for the total area under the curve (AUC) for each vaccinated cohort, compared with the placebo recipients. AUC was calculated for each participant relative to baseline (day 0) total antigen-specific CD4-positive T-cell response. The Bonferroni method was used to account for multiple comparisons, where p<0·0125 (four tests) was considered significant. Responses in individual vaccinees are shown in the appendix. (C) Cumulative total CD4-positive T-cell responses to ID93-specific antigens. Each bar represents the median total CD4-positive T-cell response of whole blood to stimulation with pools containing Rv1813, Rv2608, Rv3619, or Rv3620 peptides. Error bars represent IQR for each stimulation. QFT=QuantiFERON TB Gold In-Tube test.

Combinatorial Polyfunctionality Analysis of Single Cells (COMPASS), using posterior probabilities that summarise for each participant and cytokine co-expression subset the evidence that the responding subset is antigen specific. This is achieved by comparing the proportion of cytokine-positive cells in the stimulated sample to the corresponding proportion in the unstimulated control sample. We also computed functionality scores that summarise the entire functionality profile into a single number. COMPASS analysis was performed using a Bioconductor package in R, as previously described.[23] Analysis was done on an intention-to-treat basis, unless otherwise noted.

The trial is registered with ClinicalTrials.gov, number NCT01927159, where the full study protocol is available.

Role of the funding source

Aeras, a funder of the study, was involved in the study design, interpretation of the data, and writing of the report. The Paul G Allen Family Foundation was involved in funding of the study. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Aug 30, 2013, and Sept 4, 2014, 227 individuals consented to participate; 213 were screened (three participants were not included as study number was already met and 11 withdrew consent before screening occurred, mostly due to relocation or demands of

	Placebo group (saline; n=12)			Cohort 1 (QFT-, 10 µg ID93 + 2 µg GLA-SE; n=9)			Cohort 2 (QFT-/+, 2 µg ID93 + 2 µg GLA-SE; n=15)			Cohort 3 (QFT-/+, 10 µg ID93 + 2 µg GLA-SE; n=15)			Cohort 4 (QFT-/+, 10 µg ID93 + 5 µg GLA-SE; n=15)			QFT-, 10 µg ID93 + 2 µg GLA-SE; n=10 (cohorts 1 and 3)			QFT+, 10 µg ID93 + 2 µg GLA-SE; n=14 (cohorts 1 and 3)		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Number dosed	12	12	11	9	9	9	15	15	15	15	15	13	15	15	13	10	10	10	14	14	12
Statistic																					
Participants with at least one adverse event	5 (42%)	5 (42%)	3 (27%)	6 (67%)	6 (67%)	4 (44%)	15 (100%)	13 (87%)	11 (73%)	14 (93%)	13 (87%)	9 (69%)	14 (93%)	13 (87%)	10 (77%)	6 (60%)	6 (60%)	4 (40%)	14 (100%)	13 (93%)	9 (75%)
Participants with at least one solicited adverse event	0	0	0	5 (56%)	5 (56%)	2 (22%)	15 (100%)	13 (87%)	8 (53%)	12 (80%)	13 (87%)	9 (69%)	10 (67%)	13 (87%)	8 (62%)	5 (50%)	5 (50%)	2 (20%)	12 (86%)	13 (93%)	9 (75%)
Severity*																					
Mild	2 (17%)	4 (33%)	2 (18%)	5 (56%)	4 (44%)	3 (33%)	9 (60%)	7 (47%)	7 (47%)	13 (87%)	8 (53%)	7 (54%)	12 (80%)	6 (40%)	8 (62%)	5 (50%)	4 (40%)	3 (30%)	13 (93%)	8 (57%)	7 (58%)
Moderate	1 (8%)	1 (8%)	1 (9%)	1 (11%)	2 (22%)	1 (11%)	6 (40%)	5 (33%)	4 (27%)	1 (7%)	5 (33%)	2 (15%)	2 (13%)	7 (47%)	2 (15%)	1 (10%)	2 (20%)	1 (10%)	1 (7%)	5 (36%)	2 (17%)
Severe	2 (17%)	0	0	0	0	0	0	1 (7%)	0	0	0	0	0	0	0	0	0	0	0	0	0
Relationship†																					
Not related	4 (33%)	3 (25%)	1 (9%)	0	0	0	0	0	0	0	0	0	2 (13%)	0	0	0	0	0	0	0	0
Related	1 (8%)	2 (17%)	2 (18%)	6 (67%)	6 (67%)	4 (44%)	15 (100%)	13 (87%)	11 (73%)	14 (93%)	13 (87%)	9 (69%)	12 (80%)	13 (87%)	10 (77%)	6 (60%)	6 (60%)	4 (40%)	14 (100%)	13 (93%)	9 (75%)
Serious adverse events	0	1 (8%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(Table 2 continues on next page)

employment), and 66 healthy, HIV-negative adults, with a median age of 25 years, were sequentially randomly assigned into consecutive cohorts (table 1, figure 1A). In total, 12 participants received placebo and 54 participants received the vaccine. All study participants received day 0 and day 28 study injections. Five participants (one placebo recipient, and two vaccine recipients each from cohorts 3 and 4) did not receive day 112 study injections due to pregnancy (n=1) or unavailability (n=4). Seven QFT-negative participants in cohort 1 (one placebo recipient and six vaccine recipients) received delayed day 28 injections (administered on day 74–117) after a pause in the study for sponsor inspection of vaccine vials. Intention-to-treat data are included in this analysis, and per-protocol immunogenicity results can be found in the appendix. Participants were followed up to study day 294, with the last study visit concluded in July, 2015.

No vaccine-related severe adverse events or serious adverse events were recorded. Most adverse events were mild or moderate in severity and only three events (0·9%), unrelated to vaccine, were graded as severe (elevated systolic or diastolic blood pressure). One serious adverse event (spontaneous incomplete abortion) was recorded in a placebo recipient (table 2, appendix). Injection site pain was reported more often in *M tuberculosis*-infected than uninfected vaccine recipients (100%, 95% CI 78·5–100 *vs* 60%, 31·3–83·2; p=0·02, relative risk [RR] 1·67, 95% CI 1·01–2·76) receiving equivalent 10 µg ID93 plus 2 µg GLA-SE dose (table 2); although the proportion of adverse events was high in both groups, there was no significant increase in the overall adverse event rate (100%, 78·5–100 *vs* 70%, 39·7–89·2; p=0·06, RR 1·43, 0·95–2·14) or injection site swelling (21%, 7·5–47·6 *vs* 0%, 0–27·8; p=0·24, RR 4·29 (0·24–infinity). Mild flu-like symptoms, resolving within 48 h, were more common in *M tuberculosis*-infected participants after the second and third vaccine doses than in uninfected participants. Placebo recipients experienced no local injection site events. The safety data suggest that escalating doses of ID93+GLA-SE had an acceptable safety profile in BCG-vaccinated *M tuberculosis*-infected and uninfected participants, with an increasing trend of adverse events as the dose or adjuvant level increased, and in individuals who were *M tuberculosis*-infected at baseline.

Next, we assessed vaccine-induced immunogenicity; we used whole blood ICS assay and flow cytometry to measure frequencies of antigen-specific IFNγ, TNF-α, IL-2, and IL-17 expressing CD4-positive and CD8-positive T cells. Responses in individual vaccine recipients are shown in the appendix. ID93+GLA-SE vaccination induced marked and rapid increases in frequencies of total cytokine-expressing CD4-positive T cells specific to Rv1813, Rv2608, Rv3619, and Rv3620 in all four cohorts, irrespective of baseline *M tuberculosis*-infection status (figure 2A). No significant increases in ID93-antigen-specific total cytokine positive CD4-positive T cell responses were seen in placebo recipients (figure 2A).

	Placebo group (saline; n=12)			Cohort 1 (QFT-, 10 μg ID93 + 2 μg GLA-SE; n=9)			Cohort 2 (QFT-/+, 2 μg ID93 + 2 μg GLA-SE; n=15)			Cohort 3 (QFT-/+, 10 μg ID93 + 2 μg GLA-SE; n=15)			Cohort 4 (QFT-/+, 10 μg ID93 + 5 μg GLA-SE; n=15)			QFT-, 10 μg ID93 + 2 μg GLA-SE; n=10 (cohorts 1 and 3)			QFT+, 10 μg ID93 + 2 μg GLA-SE; n=14 (cohorts 1 and 3)		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
(Continued from previous page)																					
Local solicited adverse events‡																					
Injection site erythema	0	0	0	0	0	0	0	1 (7%)	0	0	2 (13%)	1 (8%)	0	2 (13%)	0	0	0	0	0	2 (14%)	1 (8%)
Injection site pain	0	0	0	4 (44%)	5 (56%)	1 (11%)	14 (93%)	12 (80%)	6 (40%)	12 (80%)	13 (87%)	6 (46%)	10 (67%)	10 (67%)	6 (46%)	4 (40%)	5 (50%)	1 (10%)	12 (86%)	13 (93%)	6 (50%)
Injection site swelling	0	0	0	0	0	0	4 (27%)	3 (20%)	0	0	2 (13%)	2 (15%)	0	4 (27%)	2 (15%)	0	0	0	0	2 (14%)	2 (17%)
Systemic solicited adverse events‡																					
Chills	0	0	0	0	0	0	0	3 (20%)	1 (7%)	0	4 (27%)	0	0	2 (13%)	4 (31%)	0	0	0	0	4 (29%)	0
Fatigue	0	0	0	0	1 (11%)	0	0	0	0	0	3 (20%)	0	0	1 (7%)	1 (8%)	0	1 (10%)	0	0	3 (21%)	0
Headache	0	0	0	1 (11%)	2 (22%)	1 (11%)	0	2 (13%)	1 (7%)	0	5 (33%)	3 (23%)	2 (13%)	6 (40%)	2 (15%)	1 (10%)	2 (20%)	1 (10%)	0	5 (36%)	3 (25%)
Myalgia	0	0	0	0	1 (11%)	0	1 (7%)	5 (33%)	2 (13%)	1 (7%)	4 (27%)	2 (15%)	3 (20%)	4 (27%)	1 (8%)	0	1 (10%)	0	1 (7%)	4 (29%)	2 (17%)
Unsolicited adverse events‡																					
Alanine aminotransferase increased	0	1 (8%)	0	1 (11%)	2 (22%)	1 (11%)	1 (7%)	0	1 (7%)	0	0	0	1 (7%)	2 (13%)	0	1 (10%)	2 (20%)	1 (10%)	0	0	0
Blood pressure diastolic increased	1 (8%)	0	0	1 (11%)	0	1 (11%)	2 (13%)	2 (13%)	1 (7%)	1 (7%)	2 (13%)	0	0	0	0	1 (10%)	0	1 (10%)	1 (7.1%)	2 (14%)	0
Injection site warmth	0	0	0	0	0	0	0	1 (7%)	0	0	1 (7%)	1 (8%)	2 (13%)	3 (20%)	0	0	0	0	0	1 (7%)	1 (8%)
Lymphadenopathy	0	0	0	0	0	0	0	0	1 (7%)	0	3 (20%)	1 (8%)	0	0	0	0	0	0	0	3 (21%)	1 (8.3%)
Malaise	0	0	0	0	0	0	0	2 (13%)	0	0	0	1 (8%)	0	2 (13%)	0	0	0	0	0	0	1 (8%)
Nausea	0	0	0	0	0	0	0	1 (7%)	0	0	0	0	0	3 (20%)	1 (8%)	0	0	0	0	0	0
Neutrophil count decreased	0	1 (8%)	2 (18%)	2 (22%)	0	0	3 (20%)	4 (27%)	3 (20%)	0	0	0	1 (7%)	1 (7%)	1 (8%)	2 (20%)	0	0	0	0	0
Tachycardia	0	0	0	0	0	0	0	1 (7%)	2 (13%)	0	2 (13%)	0	0	1 (7%)	0	0	0	0	0	2 (14%)	0
Upper respiratory tract infection	2 (17%)	1 (8%)	0	0	0	0	0	0	0	0	1 (7%)	0	2 (13%)	0	0	0	0	0	0	1(7%)	0
Vomiting	0	0	0	0	0	0	0	0	0	0	0	0	0	2 (13%)	0	0	0	0	0	0	0

QFT=QuantiFERON. *Participants counted once at highest severity level for each dose. †Participants counted once at strongest relationship for each dose. ‡Includes adverse events reported by two or more participants receiving ID93 + GLA-SE after any dose in any cohort.

Table 2: **Adverse events by dose and number of administrations in each cohort, and by QFT status in participants receiving an equivalent dose (10 μg ID93 + 2 μg GLA-SE)**

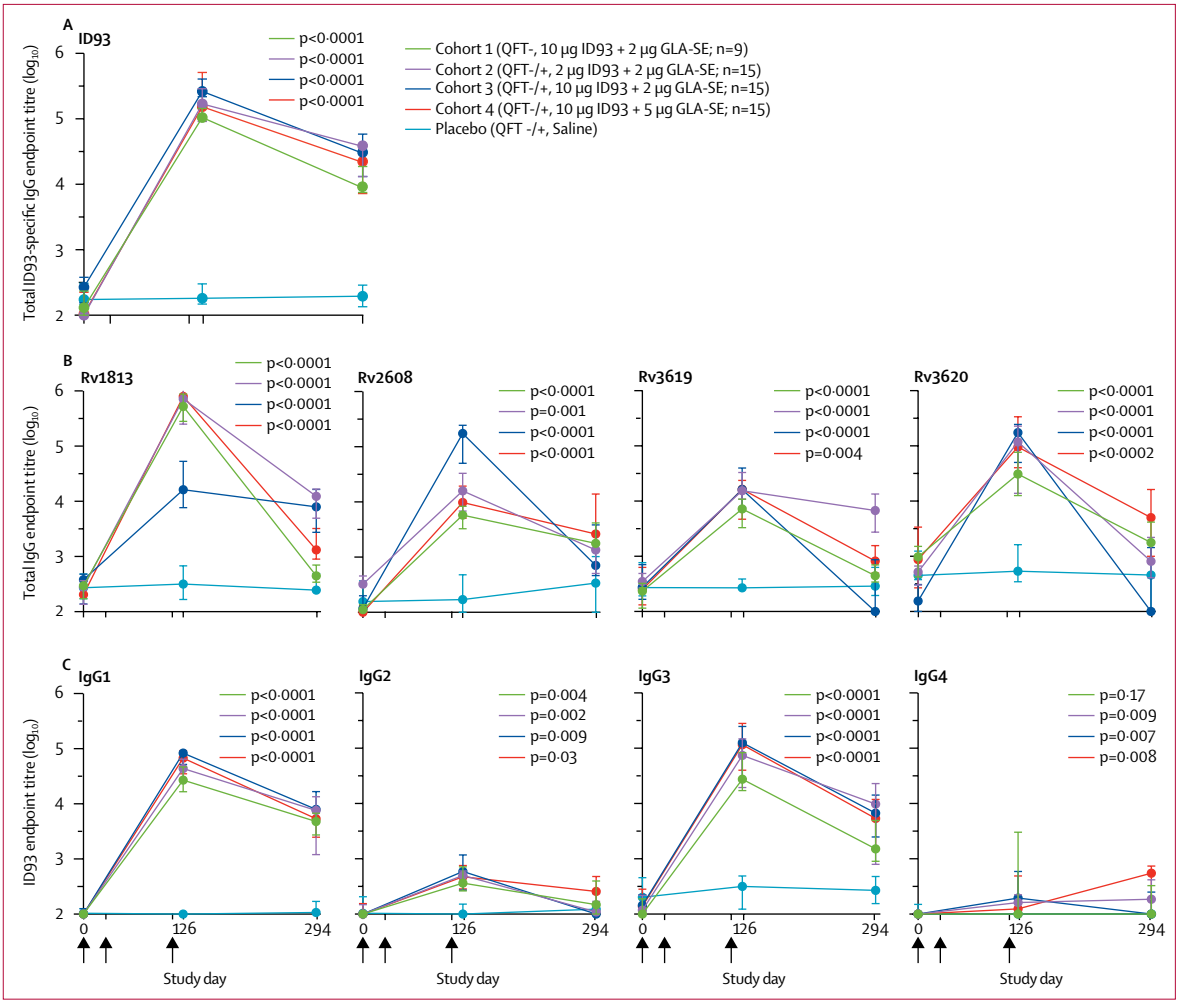

Figure 3: **Median humoral responses induced by vaccination, stratified according to study cohort**

(A) Total ID93-specific IgG antibody endpoint titre responses, measured in diluted serum by ELISA. (B) Total IgG endpoint titre responses for each antigen component. (C) Endpoint titres for IgG subclasses specific for ID93. For all graphs, bars represent IQRs, and arrows indicate days of vaccine or placebo administration. Mann-Whitney p-values are shown for the total area under the curve (AUC) for each vaccinated cohort, compared with the placebo recipients. AUC was calculated for each participant relative to baseline (day 0) endpoint titre. Uncorrected p values are shown. The Bonferroni correction method was used to account for multiple comparisons, where p<0·0125 (four tests) was considered significant.

We analysed longitudinal frequencies of antigen-specific cytokine-expressing CD4-positive T cells by calculating the response AUC for each participant. Despite the increase in Rv1813-specific CD4-positive T-cell responses after ID93+GLA-SE vaccination, comparison of response AUCs showed that only vaccinees in cohort 2 had higher Rv1813-specific responses than placebo recipients. By contrast, vaccinees in all four cohorts had significantly increased longitudinal CD4-positive T-cell responses to Rv2608 compared with placebo recipients (figure 2A), irrespective of antigen or adjuvant dose or baseline QFT status. Longitudinal Rv3619 and Rv3620-specific CD4-positive T-cell responses were also significantly elevated in cohorts 2, 3, and 4, but those in cohort 1 were not significantly increased after adjustment for multiple comparisons. These data suggest that responses to Rv1813, Rv3619, and Rv3620 were readily boosted by ID93+GLA-SE in individuals with pre-existing *M tuberculosis* infection, whereas induction of these responses in *M tuberculosis*-uninfected individuals was less pronounced. In all four cohorts, Rv1813 and Rv2608-specific CD4-positive T-cell responses peaked after two vaccine administrations, irrespective of dose level, and were not appreciably boosted by a third administration.

Frequencies of cytokine-positive CD8-positive T-cell responses against any of the vaccine antigens were very low in all cohorts (median frequencies <0·01%) and not significantly higher than those in placebo recipients (figure 2B). Consequently, subsequent in-depth analyses focused on CD4-positive T-cell responses. CD4-positive and CD8-positive T-cell responses for individual participants are shown in the appendix.

Median total CD4-positive Th1 cytokine responses to all antigens peaked at day 42 in all vaccinated groups

(figure 2C). The total CD4-positive T-cell response to vaccination was generally dominated by Rv2608 and Rv3619-specific cells, while many vaccinees also responded to Rv3620. In cohorts 2, 3, and 4, a particularly high-magnitude CD4-positive T-cell response was recorded against Rv2608, followed by near equivalent responses to Rv3619 and Rv3620 (figure 2C).

ID93-specific IgG responses also increased significantly after the three ID93 + GLA-SE vaccinations in participants from all four cohorts, and were maintained well above pre-vaccination levels and those in placebo recipients at the end of the study (figure 3A). Humoral responses were detected against all four antigens (figure 3B) and were dominated by IgG1 and IgG3 (figure 3C). In contrast to T-cell responses, we recorded high IgG concentrations against Rv1813 (figure 3B and appendix).

Our results show that ID93 + GLA-SE is immunogenic and that neither escalation of the protein nor the adjuvant dose resulted in significantly different responses.

To assess whether ID93 + GLA-SE induced sustained memory T-cell responses, we compared magnitudes of total cytokine-producing CD4-positive T-cell responses 6 months after the final vaccine administration to baseline responses (appendix). Antigen-specific CD4-positive T-cell responses to all four antigens were maintained at frequencies that significantly exceeded the pre-vaccination responses in cohorts 2 and 4. Only Rv2608-specific CD4-positive T cells were maintained at frequencies that exceeded the pre-vaccination response in *M tuberculosis*-uninfected cohort 1 participants. COMPASS functionality scores, representing posterior probabilities that responses were antigen-specific,[23] indicated durable Rv2608-specific CD4-positive T cells in all cohorts except cohort 1 (appendix). While low magnitude Rv1813-specific responses were recorded, functionality scores suggested durability in cohorts 1, 2, and 4. Rv3620-specific responses were only maintained to day 294 in Cohort 4.

In these assays, frequencies of IL-17-expressing CD4-positive T cells were low or undetectable (appendix). Consequently, we focused on Th1 cytokine (IFNγ, TNF-α or IL-2, or both) co-expression patterns in subsequent analyses. Participants in cohorts 1 and 3 received an equivalent dose of vaccine, allowing analysis of vaccine-induced immune responses stratified by QFT status. Rv2608-specific and low-level Rv1813-specific CD4-positive T-cell responses in both groups were mainly comprised of TNF-α+IL-2+ or single IL-2-expressing cells (appendix), although IFNγ+IL-2+TNF-α+ CD4-positive T cells were also induced in *M tuberculosis*-infected participants. *M tuberculosis*-infected vaccinees generally had much broader response profiles dominated by IFNγ+IL-2+TNF-α+ cells. Rv3619 and Rv3620-specific CD4-positive T-cell responses in *M tuberculosis*-uninfected participants were low (appendix), precluding definitive analysis of cytokine co-expression. In *M tuberculosis*-infected participants, Rv3619 and Rv3620-specific CD4-positive T-cell responses were dominated by IFNγ-expressing subsets, particularly IFNγ+IL-2+TNF-α+ cells (appendix). Vaccination resulted in antigen-specific increases in polyfunctionality (appendix). Additionally, we noted higher post-vaccination IgG1 and IgG3 responses in *M tuberculosis*-infected individuals against Rv3620 and Rv3619, compared with uninfected individuals, suggesting natural priming by *M tuberculosis* infection (appendix).

The degree of T-cell differentiation could affect cellular functionality, memory, cell trafficking, and the capacity to control *M tuberculosis*.[24,25] Thus, we aimed to assess the degree of antigen-specific CD4-positive T cell differentiation after vaccination with ID93 + GLA-SE. Cytokine co-expression profiles show the degree of CD4-positive T-cell differentiation; IL-2 or TNF-α expression in the absence of IFNγ reflects early differentiated central memory cells, while IFNγ co-expression with IL-2 or TNF-α marks more differentiated effector memory or, in the absence of IL-2 or TNF-α, terminally differentiated effector cells.[25] Pre-vaccination responses of individuals mainly comprised the IFNγ+IL-2+TNF-α+ subset and were more common in *M tuberculosis*-infected vaccine recipients, especially for Rv2608 and Rv3619-specific cells (appendix). After vaccination, peak Th1 responses were recorded at day 42 in many vaccinees. Again, Rv2608 and Rv3619-specific CD4-positive T cells were most prominent and comprised all Th1 cytokine co-expression subsets. These were maintained up to day 294 in most *M tuberculosis*-infected participants.

We also sought to explore the degree of differentiation of antigen-specific CD4-positive T cells further, and calculated the previously defined functional differentiation score (FDS),[24] which represents the proportion of antigen-specific Th1 cytokine-positive CD4-positive T cells expressing IFNγ as a ratio of antigen-specific CD4-positive T cells not expressing IFNγ (ie, expressing TNF-α or IL-2). In *M tuberculosis*-infected and uninfected vaccinees receiving equivalent vaccine doses, Rv2608-specific CD4-positive T cells had low FDS, suggesting T cells in early stages of differentiation. Rv1813 and Rv3619-specific CD4-positive T cells were not sufficiently frequent in *M tuberculosis*-uninfected participants to definitively measure differentiation. However, in *M tuberculosis*-infected participants, Rv3619 and Rv3620-specific CD4-positive T cells had markedly higher FDS than Rv2608 and Rv1813-specific cells, suggesting more differentiated cells to the former two antigens (appendix). Strikingly, FDS correlated with the proportion of CCR7-CD45RA- effector memory CD4-positive T cells specific for each antigen, especially during the response peak at day 42 (appendix), further supporting the differentiation profiles of these T-cell responses.

Vaccination of *M tuberculosis*-infected participants thus resulted in greater Rv3619 and Rv3620-specific CD4-positive T-cell differentiation and effector memory phenotypes, compared with uninfected participants (appendix). Taken together, we recorded broad diversity in the differentiation of ID93 + GLA-SE induced CD4-

positive T-cell responses against each antigen in participants with and without underlying *M tuberculosis* infection. The higher FDS and effector memory phenotypes recorded in *M tuberculosis*-infected vaccine recipients, relative to the uninfected group, suggested that vaccination does not strongly drive T-cell differentiation in uninfected participants, nor markedly modulate already well differentiated CD4-positive T-cell responses induced by natural *M tuberculosis* infection.

Discussion

This study is the first to report the effect of ID93 + GLA-SE vaccination in human beings. The vaccine showed a favourable safety profile for all antigen and adjuvant dose levels administered up to three times. Although increased injection site reactogenicity and more frequent flu-like symptoms were recorded in *M tuberculosis*-infected compared with uninfected recipients receiving equivalent doses of ID93 and GLA-SE, such reactions were transient. Overall, the safety and reactogenicity profile of ID93 + GLA-SE was acceptable in all cohorts.

Three vaccinations with ID93 + GLA-SE induced high-magnitude and sustained antigen-specific CD4-positive T cell and IgG responses. The antigen-specific CD4-positive T-cell response peaked 2 weeks after the second injection, although the third vaccination also boosted the T-cell response. The peak CD4-positive T-cell response and cytokine co-expression profiles, which indicate T-cell differentiation and effector function, varied between the different antigenic components of ID93 and by *M tuberculosis* infection status. Rv2608 was the most immunodominant T-cell antigen, followed by both Rv3619 and Rv3620. T-cell responses to Rv1813 were of low magnitude overall although IgG responses to this antigen were the most commonly reported. This complexity highlights the need to measure immune responses against each vaccine antigen individually to capture the diversity in responses in individuals living in a setting endemic for tuberculosis. Our data clearly suggest that vaccine-induced responses could be modulated by natural infection with *M tuberculosis* or non-tuberculous mycobacteria. The magnitude and kinetics of these responses differed between QFT-positive and QFT-negative vaccinees, likely because of previous sensitisation by *M tuberculosis*. In *M tuberculosis*-infected individuals, Rv3619-specific and Rv3620-specific CD4-positive T cells rapidly expanded after a single administration of ID93 + GLA-SE, with subsequent administrations having little added effect at the study timepoints tested here.

We noted that Rv3620 and Rv3619-specific T cells displayed effector memory phenotype and high FDS, consistent with advanced T-cell differentiation, whereas Rv2608 and Rv1813 displayed lower FDS and central memory phenotypes. These data are consistent with higher levels of Rv3620 and Rv3619 antigen presentation relative to the other two antigens in individuals with *M tuberculosis* infection (and relative to individuals without *M tuberculosis* infection), suggesting that Rv3620 and Rv3619 are chronically expressed in vivo by *M tuberculosis*, as has been reported for ESAT-6.[24] Moguche and colleagues[24] have reported that persistent, high-level expression of ESAT-6 during *M tuberculosis* infection induces more differentiated CD4-positive T-cell response profiles than those against Ag85B, which is highly expressed only during acute *M tuberculosis* infection. Similar response profiles were recorded in South Africans from the same study population as those studied in the present ID93 + GLA-SE trial, who were vaccinated with the ESAT-6 and Ag85B-containing H1:IC31 tuberculosis vaccine candidate.[24] Because Rv3619 and Rv3620 are members of the Esx-1 system, like ESAT-6, we believe that our data suggest that T cells specific for these two antigens are exposed to higher levels of antigen in vivo, thus driving greater T-cell differentiation relative to T cells specific for Rv2608 or Rv1813.

Escalation of the ID93 + GLA-SE dose did not result in greater magnitude of CD4-positive T-cell or antibody responses at the evaluated timepoints, suggesting that the lowest vaccine dose might be suitable for subsequent efficacy studies, which would allow dose sparing for future implementation.

Although IFNγ-producing CD4-positive T cells are necessary to mediate control of *M tuberculosis* replication in animal models, induction of such Th1 responses is not sufficient to eradicate or markedly protect against pulmonary *M tuberculosis* infection.[26,27] Furthermore, despite inducing IFNγ-producing antigen-specific CD4-positive T cells, MVA85A, the only new infant tuberculosis vaccine candidate tested for efficacy in the past 50 years, did not offer additional protection beyond that of BCG.[28] These studies suggest that attributes of the CD4-positive T-cell response other than magnitude and IFNγ expression could contribute to protective immunity against *M tuberculosis*. These other attributes could include the antigenic target, induction of other groups of the adaptive immune response, such as functional antibodies or the quality, function, and differentiation of the T-cell response, which are likely to effect the tissue location and kinetics of responses.[10,11]

How functional attributes of antigen-specific CD4-positive T cells will be affected by events, such as recent or underlying subclinical tuberculosis disease, is presently unknown. Studies have reported a skewing towards IFNγ-expressing, monofunctional effector T cells, terminal differentiation and possible immune exhaustion in individuals with active tuberculosis disease.[29] We only evaluated functional states of peripheral CD4-positive T cells, which might differ from those in the lung, where antigenic stimulation might drive differentiation. Non-human primate models suggest that ID93 + GLA-SE could have application also as a therapeutic vaccine,[20] and such questions will be addressed in an ongoing phase 2a study to test ID93 + GLA-SE safety and immunogenicity in preparation for efficacy studies to prevent recurrent

tuberculosis in individuals who have recently completed treatment for active tuberculosis (NCT02465216).

In this dose-escalation study, the safety profile of ID93+GLA-SE in both *M tuberculosis*-infected and uninfected people was acceptable in all cohorts, with up to three administrations of vaccine. Given the interaction of *M tuberculosis* infection with the kinetics, magnitude, and quality of CD4-positive T-cell responses, our data suggest that two doses of vaccine are preferable over a single administration in a tuberculosis endemic region where vaccine recipients are likely to be a mix of both *M tuberculosis*-infected and uninfected people. Such a strategy would avoid the need for pre-vaccination QFT screening.

Our study had limitations, including effects of several missed or out-of-window visits, particularly in the *M tuberculosis*-uninfected participants of cohort 1, which might have affected interpretation of response kinetics. While CD4 T-cell responses to Rv3619 and Rv3620 in cohort 1 seem low compared with cohorts 2–4, the delay in administration of the second injection to some cohort 1 participants did not affect the kinetics of Rv1813-specific or Rv2608-specific responses. Unfortunately, small sample sizes within each cohort, and the distribution of infected and uninfected participants within, confounds interpretation of the per-protocol analysis. This phase 1 study was designed for assessment of the primary outcome of safety; more definitive assessment of immunological outcomes will be addressed in larger phase 2 studies. Follow-up of participants in the study was also restricted to 6 months after the last vaccine administration, which hindered measurement of durability of vaccine-induced memory cells beyond this period.

While we currently lack a vaccine mediated immune correlate of protection for tuberculosis in human beings, pre-clinical findings support advancement of this candidate.[17–20,30] The implications of the diversity of co-expression of different Th1 cytokine combinations, and therefore the memory differentiation and functionality of ID93-specific CD4-positive T cells, for protective efficacy of the vaccine can only be established in an efficacy trial. However, in view of the unknown T-cell functions and differentiation necessary for protective immunity against *M tuberculosis*, we can only measure vaccine take in such early trials. This study aimed to support selection of a dose for further vaccine development. Dose selection was based mainly on safety, but also on measurement of the immunological signal—a Th1 response—that we consider the most appropriate for vaccination against tuberculosis. We propose that a sustained vaccine-induced CD4-positive T-cell response with a broad, diverse functionality profile, such as that provided by the four different antigens in ID93+GLA-SE, is more likely to include protective responses than a narrow response. In addition, vaccination elicited IgG1 and IgG3, subclasses thought to have increased Fc-mediated functional capacity.[11] These encouraging immunogenicity data, coupled with findings of safety in *M tuberculosis*-infected individuals, provide a strong rationale for further testing of this vaccine in efficacy trials in tuberculosis endemic countries.

Contributors

AP-N, MT, LJ, RE, AMG, WAH, SGR, RNC, TJS, and MH conceived of the study design. MT, WAH, and MH developed and recruited cohorts that were used in this study. MT, HG, and AK-KL provided clinical help. ES, JV, SM, and NB collected data used in experiments. AP-N, MT, ES, TAD, MM, JV, RE, RNC, TJS, and MH analysed the data. AP-N, MT, TAD, MM, JV, RE, AMG, RNC, TJS, and MH contributed to interpretation of results. AP-N, MT, TJS, and MH wrote the manuscript. All authors had full access to the data, and reviewed, revised, and gave final approval of the manuscript before submission.

Declaration of interests

This work was supported by co-funding from Aeras and the Paul G Allen Family Foundation. SGR is a founder of, and holds an equity interest in, Immune Design Corp, a licensee of certain rights associated with GLA. MH reports clinical trial grants from Aeras to the University of Cape Town during the conduct of the study. AMG reports grants from the Bill & Melinda Gates Foundation and DFID (UK Government) during the conduct of the study; non-financial support and other support was provided by GSK and Sanofi Pasteur, outside of the submitted work. All other authors declare no competing interests. Parts of this work were presented at the Keystone Symposium on Translational Vaccinology for Global Health (S1), in London, England, on Oct 27, 2016.

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